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Cadmium, Lead and Zinc in Growing Rats Fed Corn Leaf Tissue Grown on Soil Amended with Sewage Sludge or Heavy Metal Salts

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Rats were fed, from weaning through 11 weeks of age, dried leaf tissue of corn plants grown on soil amended with regular NPK fertilizer (150-22-62), with 33.6 and 67.2 metric ton/ha of sewage sludge or with salts of cadmium (10kg/ha), lead (25kg/ha), and/or zinc (50kg/ha). A very high proportion of the cadmium (cd) consumed was eliminated in the feces. Only in rats fed diets containing leaf tissue from plants grown on soil to which $CdCl_2$ salt or the high level of sludge had been added did the metal accumulate in significantly greater quantity than in rats fed a standard diet without leaf tissue. Most of the carcass accumulation of Cd could be accounted for by that in the liver and kidneys. The proportion of dietary zinc (Zn) that was excreted in feces was less than that for Cd, indicating that more Zn was absorbed into the body. There was no correlation between intake and accumulation of Zn in the tissues, however, so that much of the absorbed Zn must have been eliminated in some way. Fecal elimination did not serve as a way to rid the body of excessive intake of lead (Pb). However, with intakes ranging from 2 to 11 mg total in this study, the carcass load did not exceed 1.1 mg of Pb. Thus absorbed Pb, like Zn, must also be eliminated efficiently. No gross signs of toxicity or of physiological impairment were observed in rats fed any of the plant tissue samples.

Introduction

Treatment and disposal of municipal wastewater and sewage sludge are major environmental problems. The need to find safe ways of disposal, and anticipation of some benefit to be gained, resulted in numerous studies involving sewage sludge as a soil treatment on agricultural lands. The potential hazard of certain heavy metals accumulating in soils from applied sludge and subsequent transfer of these metals through the food chain to man is recognized (*1*, *2*). Hinesly et al. (*3*) reported that furrow irrigation of corn plots with heated, anaerobically digested sludge led to increases in cadmium and zinc in leaf and grain tissues that were related to rate of application. Sludge treatment of strip-mine soil increased yields of corn without causing significant differences in heavy metal content of the grain (*4*). In subsequent studies from the same laboratory, Garcia et al. (*5*) investigated translocation and accumulation of heavy metals in various tissues of corn plants. Generally, the highest concentrations of metals were found in roots and leaves and the lowest in grain and cob. Increases in zinc, copper, chromium and cadmium in leaf tissue occurred as a result of sludge application, with the increase in cadmium being greatest. Furr et al. (*6*) grew plants representing major classes of edible garden crops on sludge amended soil in pots. Of the 42 elements determined, 12 were found at higher concentrations in sludge treated versus untreated areas with at least three of the

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crops grown; beans, cabbage, carrots, millet, potatoes, and tomatoes. Cadmium and zinc were found at particularly high concentrations in cabbage, millet and onions. Chaney (*7*) and Page (*2*) have reviewed the effects of soil characteristics on the extent of uptake of such elements by plants.

We fed grains of corn, sorghum, and soybeans that were grown on soils treated with sewage sludge to weanling rats (*8*). Differences in metal content of liver and kidney tissues associated with type of soil treatment were found only for Mn, Cu and Fe and even for these elements all values were within normal ranges. Cd, Ni and Pb were not present at detectable levels in the grains or rat tissues. Melsted et al. (*9*) used heavier applications of sludge than we did and found Cd in corn grain and in liver, kidney and duodenum tissues of pheasants fed the grain. Cd load in plant and bird tissues increased with increasing rates of sewage sludge applied to the land.

Leaf tissue of plants grown on sludge amended soil accumulate higher concentrations of heavy metals than do the grains or fruits of the plants. Transfer of these metals, especially cadmium and zinc, to mammals fed the crop components has been reported. Cadmium was increased in kidneys of guinea pigs fed Swiss chard (*10*) but composting the sludge seemed to reduce the rate of transfer of metal to the plant (*11*). The concentration of cadmium and zinc in lettuce was closely correlated with the element levels in the respective municipal sludges on which they were grown. Cadmium was significantly higher in kidneys of mice fed the sludge grown lettuce than in those fed lettuce grown on soil alone (*12*). In rats fed turnip greens grown on soil treated with sewage sludge, cadmium level of liver and kidney was increased in proportion to the rate of application of sludge to the soil (*13*). Heffron et al. (*14*) fed silage corn grown on soil amended with municipal sludge to sheep. Cadmium was higher in liver, kidney, spleen and muscle, and zinc was higher in muscle of the animals fed the sludge grown corn than in those fed the control corn.

In the experiment reported here, transfer of heavy metals through plant to animal from a municipal sewage sludge was compared with that of metals derived from inorganic salts applied to the soil in the field. Leaves of corn plants grown on field plots treated with sludge and salts of cadmium, lead and zinc were fed to rats for eight weeks. Determination of the amount of the elements in the feces indicated the extent of absorption of the metals. Accumulation in total carcass and specific tissues was also assessed.

Materials and Methods

Corn (*Zea mays* L.) "Pioneer 3009" was grown on a Cecil sandy loam (clayey, kaolinitic, thermic, Typic Hapludult) soil which had received two rates of sewage sludge applied to the soil and incorporated. The sewage sludge was from an industrial and a residential municipal, secondary treatment digested sewage sludge plants. The rates were 33.6 and 67.2 metric ton/ha applied over a 4-year period. Cadmium, lead or zinc was applied as a sidedress solution (500 mg/6.1 m row) to the soil surface along the row when corn plants were approximately 1.5 m high. Cadmium was applied at the rate of 10 kg/ha as $CdCl_2$, lead at the rate of 25 kg/ha as $Pb(CH_3COO)_2$ and Zn at the rate of 50 kg/ha as $ZnSO_4$.

Whole plant samples (above ground) were taken from the sewage sludge and heavy metal treated plots and divided into leaves, stalk and ear components when the plants had reached the "dent" stage of maturity for the corn grain. Samples were dried at 70°C in a forced-air oven, ground to pass a 0.5 mm mesh screen and samples stored in plastic containers for analysis and feeding trails.

Dried corn leaf tissue was incorporated as 15% of the total weight of rat diets (Table 1). Casein (supplemented with methionine), a complete vitamin mixture, and a complete mineral mixture were added at the same concentration as that used in the standard diet so that essential nutrients were provided at required levels without reliance upon those contained in the corn leaf tissue. Soybean oil was added at 20% of the diets to compensate for the nondigestible carbohydrate and ash of the plant material by increasing the caloric density of

Table 1. Composition of diets.

Ingredient	Content of component, g	
	Standard diet	Test diet
Corn leaves[a]	–	150
Casein	156	156
1-Methionine	2	2
Vitamin mixture[b]	22	22
Salt mixture[c]	35	35
Soybean oil	200	200
Sucrose	275	225
Corn starch	260	210
Cellulose[d]	50	–

[a]Dried and ground to pass 0.5 mm mesh screen.
[b]Vitamin Diet Fortification Mixture; ICN Nutritional Biochemicals, Cleveland, Ohio.
[c]William & Briggs, modified; Teklad Test Diets, Madison, Wisconsin.
[d]Sulkafloc.

the diet. Leaf material was replaced in the standard diet with 5% each of cellulose, sucrose, and corn starch.

Weanling rats were maintained on the standard diet for the initial 48 hours after arriving in the laboratory and then sorted into eight groups of ten animals each on the basis of body weight. One group was fed the standard diet, and one was fed the diet containing each lot of corn leaves for eight weeks. The rats were housed individually in stainless steel cages with wire mesh floors and provided with diet and deionized water ad libitum. Feces were collected each morning and stored at -5°C for subsequent analysis. Feed intake was monitored for the entire feeding trial and rats were weighed at weekly intervals.

At the end of the feeding period, half of the rats in each group were given a lethal dose of pentobarbital intraperitoneally. After death of the rat, food particles and other debris were removed from the fur by vacuuming. The peritoneal cavity was opened and the GI tract and its contents, from the proximal end of the esophagus to the anus, were removed, taking care that no blood loss occurred. The remaining part of the rat, hereafter referred to as carcass, was disintegrated by digestion in nitric acid. The fat, which rose to the top of the digestion vessel and solidified after cooling, was discarded, and the aqueous portion was made to volume from which aliquots were taken for mineral analysis.

The other rats in each diet group were anesthetized with pentobarbital and exsanguinated by severing the abdominal aorta. The liver, kidneys, heart and testes were removed and freeze-dried for subsequent analysis.

Diets, leaf tissue and animal tissues were digested with nitric and perchloric acids and inorganic elements were determined by flame atomic absorption, except for Cd which was determined by flameless atomic absorption. Data were subjected to analyses of variance and differences among means were evaluated by Duncan's (*15*) multiple range test where appropriate.

Results and Discussion

Slight burn was evident on the lower leaves of the corn on the Cd- and Zn-treated plots, but no visual effects were noted for the Pb-treated plots or the sewage sludge-treated plots. The burn effects on the Cd- and Zn-treated plots did not appear to influence subsequent plant growth or physiological development.

The data of Table 2 show growth performance and gross body composition of rats fed the diets containing the corn leaf tissues and those of rats fed a standard diet of purified ingredients. Growth and feed efficiency (weight gained/feed consumed) were both reduced by incorporation of the plant material into the diets. These effects were probably due to the nature of the corn leaf itself rather than to the prior soil treatment since none of the sludge or salt treatments gave effects different from that of the normal NPK fertilizer treatment.

The weight of dry fecal material produced was markedly increased by addition of the plant tissue to the diets. The increase in fecal mass from rats

Table 2. Growth performance and gross body composition of rats fed diets containing leaf tissue from corn plants grown on soil amended with sewage sludge or inorganic salts.

		Soil treatment[a]						
	Standard	NPK	LSS	HSS	Zn	Pb	Cd	Mix
Feed intake, g[b]	1060de	961^{f}	1042de	988^{ef}	987^{ef}	1052de	1049de	1088^{d}
Final weight, g	471^{d}	413^{e}	448de	421^{e}	424^{e}	450de	440de	445de
Feed efficiency[c]	0.391^{d}	0.370^{ef}	0.375^{e}	0.364^{ef}	0.371^{ef}	0.373^{e}	0.365^{ef}	0.357^{f}
Feces, dry weight, g[b]	106^{f}	142^{e}	152de	154de	150de	154de	150de	159^{d}
Feces, % of feed intake	10	15	15	15	15	15	14	15
Liver weight, g	19.5	17.2	17.6	17.8	15.4	18.1	17.1	18.1
% body weight	4.1^{de}	4.1^{de}	3.9^{ef}	4.3^{d}	3.7^{f}	3.9^{ef}	3.9^{ef}	4.0^{de}
Kidney weight, g	3.0	2.9	3.1	3.0	2.9	3.0	2.9	3.2
% body weight	0.64^{f}	0.71^{de}	0.69^{def}	0.73^{d}	0.69^{def}	0.65^{ef}	0.67^{ef}	0.71^{de}
Heart weight, g	1.2	1.1	1.2	1.2	1.1	1.2	1.2	1.3
% body weight	0.26	0.27	0.26	0.28	0.28	0.26	0.27	0.28
Testes weight, g	3.3	3.2	3.3	3.2	3.1	3.2	3.0	3.3
% body weight	0.69	0.77	0.73	0.76	0.76	0.71	0.69	0.73

[a]Soil treatments: NPK, regular fertilizer; LSS and HSS, 33.6 and 67.2 metric tons/ha of sewage sludge, respectively; Zn, 50 kg/ha as $ZnSO_4$; Pb, 25 kg/ha as $Pb(CH_3COO)_2$; Cd, 10 kg/ha as $CdCl_2$; and Mix, Zn + Pb + Cd at the rates indicated. Numbers in a row not followed by a common supercript letter are significantly different at $p \leq 0.05$.

[b]Feed intake and feces excreted during the 8-week feeding trial.

[c]Feed efficiency is weight gain divided by feed intake.

fed the diets containing corn tissue over that from rats fed the standard diet accounts for about 40% of the weight of leaf tissue ingested.

Organ weights and proportions of body weight were all within normal ranges for liver, kidney, heart and testes of rats fed the diets containing corn leaf tissue. Liver and kidney percentages of body weight tended to be somewhat higher for rats fed diets with leaf material from corn grown on plots treated with the high rate of sewage sludge than those fed loaf tissue produced with NPK fertilizer.

The relationships between dietary intake and fecal output of the elements cadmium, zinc, and lead are shown in Figure 1*A*, 1*B* and 1*C*, respectively. A very high proportion of the cadmium ingested passed out of the body without being absorbed. This is indicated by a regression coefficient and a correlation coefficient each of which approaches a value of one. Furthermore, although all rats fed diets containing corn leaf tissue consumed 15 μg or more of cadmium during the 8-week study, only those fed tissue from plants grown on plots treated with cadmium salt accumulated total carcass loads of more than 1.5 μg of cadmium (Table 3). For the rats fed diets containing leaves from corn grown on plots amended with the salt mix or with cadmium alone, the difference between intake and fecal excretion of cadmium amounted to about 120 and 360 μg of cadmium, respectively. However, the increase in carcass load of the element over that of rats fed the corn grown on the plots treated with NPK fertilizer was only 2.4 and 4 μg, respectively. Thus, in these cases in which a significant amount of dietary cadmium was not accounted for by that in the feces, much of what was not absorbed must have been eliminated through the kidneys, or been present in the tissues of the GI tract which were not included in the portion of the carcass analyzed. The data of Table 3 showing cadmium content of total carcass and of selected rat tissues are listed in descending order of level of dietary intake of the element. These data clearly show the strong tendency of liver and kidney to accumulated cadmium with increasing dietary intake. In fact, a large proportion of the total carcass accumulation associated with cadmium additions to the plots on which the corn plants were grown is accounted for by the excess of cadmium in these two organs. Although total carcass load of rats fed leaf tissue grown on HSS treated plots was not significantly greater than that of rats fed NPK produced leaves, cadmium level of kidney was more than four times and of liver three times greater in the former than in the latter group of animals. Heart

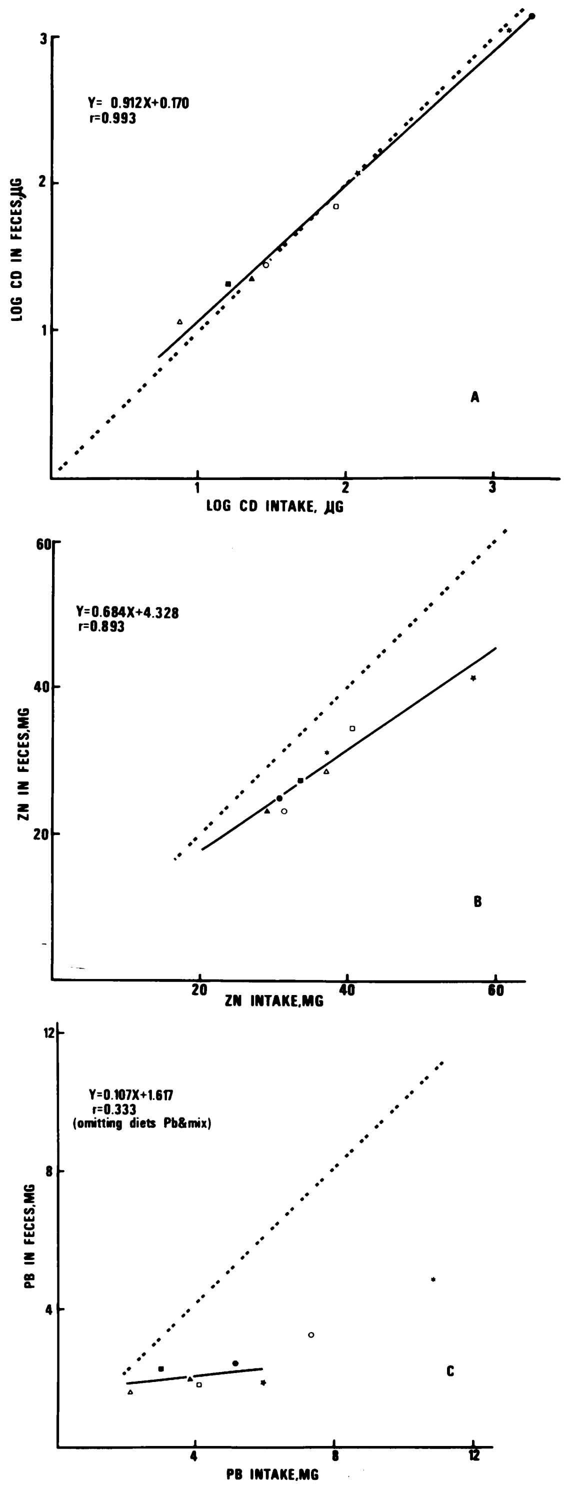

FIGURE 1. Relationship of fecal excretion to dietary intake of cadmium (*A*), zinc (*B*) and lead (*C*) by rats fed (Δ) a standard diet and diets containing corn leaf tissue grown on soils treated with (▲) NPK, (■) LSS, (*) HSS, (□) Zn, (O) Pb, (●) Cd and a mix of (*) Zn, Pb and Cd. The dotted lines represent hypothetical situations of excretion equal to intake.

Table 3. Cadmium (mean ± standard deviation) in tissues of rats fed diets containing leaf tissue from corn plants grown on soil amended with sewage sludge or inorganic salts.

	Total tissue content, ng[b]				
Diet[a]	Carcass	Liver	Kidney	Heart	Testes
Standard	1081 ± 810[c]	67.8 ± 3.9[c]	16.4 ± 5.6[d]	3.20 ± 0.96[ce]	6.25 ± 3.88[e]
Zn	1090 ± 130[c]	73.9 ± 25.2[c]	42.9 ± 11.3[d]	3.90 ± 1.64[e]	5.25 ± 2.24[ce]
NPK	1270 ± 410[c]	84.7 ± 17.9[c]	39.6 ± 3.0[d]	1.70 ± 1.01[d]	5.35 ± 1.84[ce]
Pb	1000 ± 95[c]	68.0 ± 16.4[c]	47.5 ± 11.0[d]	3.15 ± 0.72[ce]	3.50 ± 1.77[c]
LSS	1230 ± 310[c]	121.3 ± 39.3[c]	72.4 ± 6.5[d]	2.05 ± 0.27[cd]	4.35 ± 0.93[ce]
HSS	1460 ± 260[c]	220.5 ± 27.8[c]	193.2 ± 29.0[c]	2.90 ± 1.31[cde]	6.20 ± 1.76[e]
Mix	3680 ± 550[e]	1074.1 ± 202.0[e]	1185.3 ± 329.7[e]	3.65 ± 0.72[e]	6.95 ± 1.75[e]
Cd	5210 ± 1560[f]	1522.7 ± 371.0[f]	1587.8 ± 245.3[f]	7.05 ± 0.86[f]	10.00 ± 1.25[f]

[a]Soil treatments; NPK, regular fertilizer; LSS and HSS, 33.6 and 67.2 metric tons/ha of sewage sludge, respectively; Zn, 50 kg/ha as $ZnSO_4$; Pb, 25 kg/ha as $Pb(CH\ COO)_2$; Cd, 10 kg/ha as $CdCl_2$; and Mix, Zn + Pb + Cd at the rates indicated. Arranged in ascending order of dietary concentration of cadmium.

[b]Values in a column not followed by a common superscript letter are significantly different at $p \leq 0.05$.

and testes of rats fed the diets with corn leaf from plants produced with the single cadmium salt treatment also display a burden of excess cadmium accumulation.

The correlation between dietary intake and fecal output of zinc is highly significant, also (Fig. 1*B*). The regression coefficient is somewhat lower than that obtained from the intake-output relationship for cadmium, indicating that more of the dietary zinc is absorbed into the body. Unlike cadmium, there are well established physiological needs for zinc. Except for the diet containing corn leaves from plants grown on the soil to which sludge was applied at the high rate, dietary zinc concentration was only two or three times the published requirement for the rat of 12 mg/kg (*16*). There were no significant differences in zinc content of total carcass or of liver, kidney, heart or testes tissues among rats fed diets containing the various samples of corn leaves or between any of these and the rats fed the standard diet.

The relationship between fecal output and dietary intake of lead was quite different from that of the other two elements considered above. For rats fed the six diets containing corn leaves from plants grown on plots without lead salt additions, correlation between intake and output was not significant. Over an intake range of about 2 to 6 mg of lead, the fecal output was about 2 mg of lead. For those rats fed the diets with leaf tissue from plants produced on plots to which the lead salt was applied, fecal output was increased but was still less than 50% of intake. The correlation between lead intake during the 8-week feeding trial and total lead content in the carcass was not significant (Table 4). The mean intake for the eight diets fed varied from 2 to 11 mg of lead, but the carcass load of the element at the end of the study ranged only from 0.7 to 1.1 mg of lead. These data suggest that, though significant amounts of lead were absorbed into the body by rats fed some of these diets, most of the absorbed lead must have been eliminated in some way.

Differences among diet means for lead content

Table 4. Lead (mean ± standard deviation) in tissues of rats fed diets containing leaf tissue from corn plants grown on soil amended with sewage sludge or inorganic salts.

	Total tissue content, μg[b]			
Diet[a]	Carcass	Kidney	Heart	Testes
Standard	970 ± 80[cd]	2.60 ± 0.60[d]	2.65 ± 0.38[e]	4.35 ± 0.14[e]
Zn	980 ± 25[cd]	4.15 ± 0.28[c]	2.45 ± 0.67[ce]	3.55 ± 0.37[cd]
NPK	970 ± 70[cd]	2.50 ± 0.94[d]	2.25 ± 0.47[ce]	4.10 ± 0.28[ce]
LSS	770 ± 80[f]	2.55 ± 0.41[d]	2.90 ± 0.38[e]	4.25 ± 0.18[e]
Cd	900 ± 30[d]	4.45 ± 0.65[c]	1.75 ± 0.43[c]	3.75 ± 0.31[cde]
HSS	710 ± 40[f]	2.85 ± 0.49[d]	2.60 ± 0.91[e]	4.40 ± 1.01[e]
Pb	1020 ± 130[ce]	4.90 ± 0.80[ce]	1.75 ± 0.63[c]	4.00 ± 0.50[ce]
Mix	1080 ± 70[e]	5.45 ± 0.85[e]	2.40 ± 0.38[ce]	3.25 ± 0.64[d]

[a]Soil treatments: NPK, regular fertilizer; LSS and HSS, 33.6 and 67.2 metric tons/ha of sewage sludge, respectively; Zn, 50 kg/ha as $ZnSO_4$; Pb, 25 kg/ha as $Pb(CH\ COO)_2$; Cd, 10 kg/ha as $CdCl_2$; and Mix, Zn + Pb + Cd at the rates indicated. Arranged in ascending order of dietary concentration of lead.

[b]Values in a column not followed by a common superscript letter are significantly different at $p \leq 0.05$.

of kidney, heart, and testes were statistically significant but were poorly correlated with level of intake or total carcass content of the element. Liver contained about 35 μg lead and the sum of this element in liver, kidney, heart and testes was less than 7% of the total in the carcass.

Under normal circumstances, the Sprague-Dawley rat will achieve more than 90% of his maximum body size by 11 weeks of age. Thus this feeding trial, which occurred during the first 8 weeks after weaning at 21 days, took place during the period of rapid growth when nutrient supply to the body is most critical. There were no gross signs of physiological impairment or physical distress in the rats fed corn leaf tissue produced on plots amended with sludge or the inorganic salts. The accumulation of heavy metals in the corn leaves caused no apparent change in the growth performance of the rats fed these plant tissues during the short, but crucial, period following weaning. The overall reduction in growth rate of rats fed corn leaf tissue, including that grown on plots treated with the usual NPK fertilizer, was due to the indigestible nature of the material itself. The rat has a fairly large caecum but cannot utilize as high dietary concentrations of cellulose as a ruminant can.

The accumulation of cadmium in soft tissues, especially liver and kidney, could result in chronic toxicity in the animal fed tissues from plants grown on sludge amended soil. It could also be a source of cadmium ingestion by man if the plant material were fed to meat animals. Before the potential hazard to man of applying municipal sewage sludge to agricultural land can be assessed, some carefully planned, long-range studies must be made. The objectives of these studies should focus primarily on preventing accumulation of toxic elements in plants grown on the treated land since the alternative of stopping their transferral to man would be far more complex. Rates of sludge application to land that are economically feasible in terms of both crop production and waste disposal should be determined. Chaney (*7*) has shown that a high ratio of zinc to cadmium in the soil às well as maintaining a soil pH near neutrality will reduce the accumulation of the latter element in plants. Other ways to decrease uptake of this and other heavy metals by plants should be investigated.

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